Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 27, 36, 44-46, 70, 72, 92-95, 103 and 105-109 are pending in the application, with claims 27, 44 and 106 being the independent claims. Claims 39 and 104 are sought to be cancelled without prejudice to or disclaimer of the subject matter therein. Claims 27 and 70 are sought to be amended. Claims 106-109 are sought to be added.

A Request for Continued Examination (RCE) is being filed concurrently herewith. Therefore, the finality of the Office Action dated October 3, 2003 should be withdrawn, and this Supplemental Amendment and Reply should be entered and considered. *See* 37 C.F.R. § 1.114(d).

Based on the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding rejections and that they be withdrawn.

Support for Amended And New Claims

Support for amended claim 27 can be found throughout the specification, for example, at page 8, line 30, through page 9, line 13, and at page 33, line 7, through page 35, line 4.

Support for new claims 106-107 can be found throughout the specification, for example at page 33, lines 9-19, at page 54, lines 4-21, and Figure 5.

Claim Rejections Under 35 U.S.C. § 102

Claims 27, 36, 39, 92-95, 103 and 104 were rejected under 35 U.S.C. § 102(b) as being anticipated by U.S. Patent No. 2,835,586 ("Peebles") and WO 95/00031 ("Getler"), as evidenced by U.S. Patent No. 6,140,121 ("Ellington"), BBL Manual of Products and Laboratory Procedures ("the BBL Manual"), and Fassolitis *et al.*, *Appl. and Env. Microbiol. 42*:200-203 (1981) ("Fassolitis"). *See* Paper No. 22, page 2[1]. Applicants respectfully traverse this rejection for the reasons set forth in Applicants' previous replies.

Solely to expedite allowance of the rejected claims and to make explicit an implicit feature of the invention encompassed by the previously presented claims, claim 27 has been amended to recite: "An agglomerated eukaryotic cell culture medium powder prepared by agglomerating a dry powder eukaryotic cell culture medium with a solvent, *wherein said powder, upon being reconstituted with water, supports the cultivation of a eukaryotic cell in vitro*." Claim 39, directed to an agglomerated eukaryotic cell culture medium subgroup powder, has been cancelled.

None of the references cited by the Examiner describe an agglomerated powder that, upon being reconstituted with water, supports the cultivation of a eukaryotic cell *in vitro*. Peebles and Getler refer to agglomerated milk and milk-like products. Agglomerated milk

[1] The Office Action dated October 3, 2003 indicated that claim 105 was rejected under § 102; however, the Examiner clarified in the Advisory Action that the inclusion of claim 105 in the rejection was unintentional and that claim 105 is allowable.

and milk-like products, upon being reconstituted with water, would not support the cultivation of a eukaryotic cell *in vitro*. As noted in Applicants' previous responses, media for culturing eukaryotic cells must be carefully formulated and must include appropriate ingredients (*e.g.*, amino acids, vitamins, lipids, growth factors, etc.). The ingredients must be present in proportions carefully determined to allow cell growth and maintenance. Furthermore, considerations such as pH and osmolality must be appropriately adjusted based on the type of cells to be cultivated. *See, e.g.*, specification at page 14, line 25, through page 15, line 29. Even culture media for relatively simple eukaryotic organisms, such as yeasts, must be carefully formulated, taking into account the particular growth requirements of the cells. *See, e.g.*, Restrepo and Jiménez, *J. Clinical Microbiol. 12*:279-281 (1980) (copy submitted herewith as Exhibit 1). Milk and milk-like products, *without any additional ingredients*, do not contain the necessary ingredients, in the appropriate quantities, to support the cultivation of eukaryotic cells.

There is nothing in either Peebles or Getler to suggest that the agglomerated milk or milk-like products mentioned therein, when reconstituted with water, would support the cultivation of a eukaryotic cell *in vitro*. Therefore, any rejection of the present claims under § 102 based on either Peebles or Getler would have to be based on a theory of inherency. In order to show inherent anticipation, however, it must be demonstrated that the allegedly inherent characteristic is *necessarily* present in the cited art. *See In re Schreiber*, 128 F.3d 1473, 1477, 44 USPQ2d 1429, 1431 (Fed. Cir. 1997). *See also Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Int'f 1990 ("In relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the

determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art.") Thus, to establish inherent anticipation of the present claims, the evidence of record must definitively show that the milk and milk-like products of Peebles and/or Getler, when reconstituted with water, *necessarily* support the cultivation of a eukaryotic cell *in vitro*. No such evidence has been presented.

Three references, in addition to Peebles and Getler, have been cited in support of the rejection under § 102: Ellington, the BBL Manual and Fassolitis. These references do not demonstrate that the milk and milk-like products of Peebles and/or Getler, when reconstituted with water, *necessarily* support the cultivation of a eukaryotic cell *in vitro*. Thus, these references are insufficient to support a rejection based on inherent anticipation.

Ellington lists "skim milk" as one of several possible *additional components* that can be included in a culture medium. *See* Ellington at column 5, lines 29-49. Ellington does not indicate or suggest that powdered skim milk, upon being reconstituted with water, can support the cultivation of eukaryotic cells *in vitro*, nor does Ellington demonstrate the cultivation of a eukaryotic cell in skim milk. Thus, Ellington does not provide evidence that the milk and milk-like products of Peebles and/or Getler, when reconstituted with water, support the cultivation of a eukaryotic cell *in vitro*.

The BBL Manual mentions "Skim Milk Powder" for use in cultivating *bacteria*. The BBL Manual also lists "Milk-Protein Hydrolysate Peptone" as "a useful tool for general *bacteriological* culture work." There is no evidence in the BBL Manual that these compounds are useful for cultivating *eukaryotic* cells, or that, when reconstituted with water, either substance would support the cultivation of a eukaryotic cell *in vitro*. Thus the BBL

Manual does not provide evidence that the milk and milk-like products of Peebles and/or Getler, when reconstituted with water, support the cultivation of a eukaryotic cell *in vitro*.

Finally, Fassolitis relates to the ability of milk products to substitute for serum in cell culture media. Fassolitis uses nonfat dry milk filtrate as *one of many ingredients* in a cell culture medium. Fassolitis does not indicate or suggest that nonfat dry milk filtrate, upon being reconstituted with water, can support the cultivation of eukaryotic cells *in vitro*, nor does Fassolitis demonstrate the cultivation of a eukaryotic cell in nonfat dry milk filtrate reconstituted with water. Thus, Fassolitis does not provide evidence that the milk and milk-like products of Peebles and/or Getler, when reconstituted with water, support the cultivation of a eukaryotic cell *in vitro*.

Since none of the cited references indicate or even suggest that the milk and milk-like products of Peebles and/or Getler, when reconstituted with water, *necessarily* support the cultivation of a eukaryotic cell *in vitro*, a rejection based on inherent anticipation cannot be established.

In view of the arguments set forth above, Applicants respectfully request that the rejections under 35 U.S.C. § 102 be reconsidered and withdrawn.

Claim Rejections Under 35 U.S.C. § 103

Claims 27, 36, 39, 70, 72, 92-95, 103 and 104 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Peebles and Getler in view of U.S. Patent No.

5,474,931 ("DiSorbo"). *See* Paper No. 22, page 8[2]. Applicants respectfully traverse this rejection for the reasons set forth in Applicants' previous replies.

The obviousness rejection is based on the Examiner's position that Peebles and Getler teach agglomerated eukaryotic cell culture medium powders. *See* Paper No. 22, page 9. As noted above, claim 27, as currently presented, specifies that the claimed agglomerated powder, upon being reconstituted with water, supports the cultivation of a eukaryotic cell *in vitro*. Neither Peebles nor Getler teach an agglomerated eukaryotic cell culture medium powder that, upon being reconstituted with water, is capable of supporting the cultivation of a eukaryotic cell *in vitro*. *See* discussion above. Moreover, DiSorbo does not teach an agglomerated eukaryotic cell culture medium powder. A *prima facie* case of obviousness cannot be established unless all of the claim elements are taught or suggested by the cited references. *See In re Royka*, 490 F.2d 981, 984-85 (CCPA 1974); *see also In re Glaug*, 283 F.3d 1335, 1341-42 (Fed. Cir. 2002); *In re Rijckaert*, 9 F.3d 1531, 1533 (Fed. Cir. 1993). Since none of the cited references teach or suggest an agglomerated eukaryotic cell culture medium powder that, upon being reconstituted with water, is capable of supporting the cultivation of a eukaryotic cell *in vitro*, a *prima facie* case of obviousness cannot be established.

In addition, a *prima facie* case of obviousness requires that the Examiner demonstrate a suggestion or motivation to combine or modify the cited references. *See In re Rouffet*, 149 F.3d 1350, 1357, 47 USPQ2d 1453, 1457-58 (Fed. Cir. 1998). There is nothing

[2] The Office Action dated October 3, 2003 indicated that claim 105 was rejected under § 103; however, the Examiner clarified in the Advisory Action that the inclusion of claim 105 in the rejection was unintentional and that claim 105 is allowable.

to suggest modifying or combining Peebles, Getler and/or DiSorbo. Thus, a *prima facie* case of obviousness cannot be established. *See* Applicants' Amendment and Reply filed on July 17, 2003, pages 10-11.

Since not all of the elements of the claims are taught or suggested by the cited references, and since a person of ordinary skill in the art would not have been motivated to combine or modify the references, a *prima facie* case of obviousness has not been established. Applicants therefore respectfully request that the rejection under 35 U.S.C. § 103 be reconsidered and withdrawn.

Conclusion

All of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding rejections and that they be withdrawn. Applicants believe that the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Supplemental Amendment and Reply is respectfully requested.

Respectfully submitted,

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NOTES

Growth of *Paracoccidioides brasiliensis* Yeast Phase in a Chemically Defined Culture Medium

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A slight modification of the chemically defined medium of McVeigh and Morton resulted in an excellent substratum for the cultivation of *Paracoccidioides brasiliensis* yeast phase.

Repeated attempts to grow the dimorphic fungal pathogen *Paracoccidioides brasiliensis* in chemically defined media have proven unsuccessful (10, 11, 13, 15). In spite of reports concerning the adequacy of two synthetic formulations (4, 6), the results of such studies have not been reproducible (10, 11). Investigators have thus been forced to cultivate the fungus in conventional complex or basal media, the latter containing one or more undefined ingredients, e.g., yeast extract or casein hydrolysate. Consequently, previous findings concerning physiological processes (10, 11), pattern of growth curves (1, 14), antigen production (3, 9, 12), and drug susceptibility (2, 15) have met with only qualified success. The yeast phase is particularly difficult to grow in liquid media, even if the media are complex (11, 13). Since this phase corresponds to the one in tissues, obtaining yeast-phase antigens and determining the physiological characteristics of that phase would be greatly

TABLE 1. *Composition of MMcM medium*[a]

Component	Amt (per liter)
Glucose	10.0 g
KH_2PO_4	1.5 g
$MgSO_4 \cdot 7H_2O$	0.5 g
$CaCl_2 \cdot 2H_2O$	0.15 g
$(NH_4)_2SO_4$	2.0 g
L-Asparagine	2.0 g
L-Cystine[b]	0.2 g
Vitamin supplement[c]	10.0 ml
Trace element supplement[d]	1.0 ml

[a] All components except the vitamin supplement were mixed, and the pH was adjusted to 7.0 with 1 N NaOH. The vitamin solution was filter sterilized and added after the remainder of the medium had been autoclaved at 121°C for 15 min and cooled.

[b] The cystine was dissolved, before addition to the remainder of the medium, by heating it in a small volume of distilled water to which 1.0 N NaOH was added dropwise until the cystine was completely dissolved.

[c] The stock vitamin solution contained, per 100 ml: thiamine hydrochloride, 6.0 mg; niacin, 6.0 mg; calcium pantothenate, 6.0 mg; inositol, 1.0 mg; biotin, 0.1 mg; riboflavin, 1.0 mg; folic acid, 10 mg; choline chloride, 10 mg; and pyridoxine hydrochloride, 10 mg.

[d] Trace element solution contained, per 100 ml: H_3BO_3, 5.7 mg; $CuSO_4 \cdot 5H_2O$, 15.7 mg; $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, 140.4 mg; $MnSO_4 \cdot 14H_2O$, 8.1 mg; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 3.6 mg; $ZnSO_4 \cdot 7H_2O$, 79.2 mg.

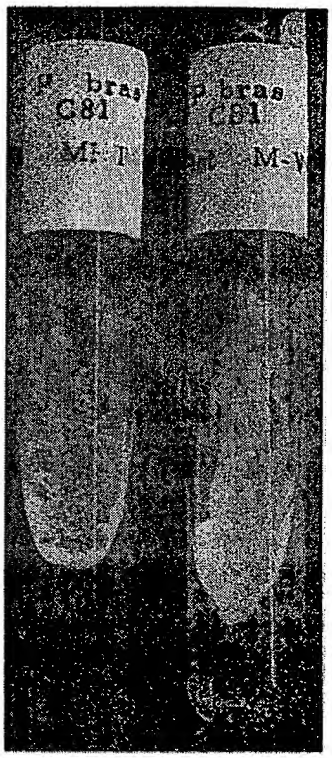

FIG. 1. *P. brasiliensis yeast-phase cultures. Tube at left, growth in complex medium. Tube at right, growth in MMcM medium.*

facilitated if the organism could be grown in a totally synthetic medium. Such studies would, in turn, facilitate other studies involving the host-parasite relationship. We report here a modification of the medium described originally by McVeigh and Morton for *Histoplasma capsulatum* (8), as reported by Levine et al. (7). Addition of four vitamins, namely, riboflavin, folic acid, choline, and pyridoxine, resulted in excellent growth of *P. brasiliensis*. This modified McVeigh-Morton (MMcM) medium can be solidified by addition of 1.3% purified agar. The components of the medium are listed in Table 1.

Studies were conducted with 20 human isolates of *P. brasiliensis* available in our collection. Stock cultures maintained at room temperature were initially reverted to the yeast phase by culturing in Kelly's hemoglobin agar (5) with incubation at 36°C for 10 days. After conversion, adaptation to solid MMcM medium was attempted by repeated transfers to fresh slants every 5 days, followed by incubation at the same temperature. After four to six such passages, all isolates produced abundant growth after 3 days. With the exception of strain B 339, growth was pasty and homogeneous, in comparison with the wrinkled colonies regularly obtained on richer media (Fig. 1). Microscopic observation revealed yeast cells with typical multiple budding. Once

TABLE 2. *Growth of P. brasiliensis yeast phase in MMcM medium*

Strain	Turbidity at h of growth[a]:		
	0	60	120
LA	50.5	9.5	1.0
C 81	52.5	24.0	3.0
MM	25.0	1.0	0.05
MTC	43.5	22.0	1.0
LS	35.5	7.0	3.8
B 339	76.0	76.0	70.0

[a] Turbidity measured by transmittance at 550 nm in a Beckman DU Spectrophotometer after growth at 36°C in liquid MMcM medium.

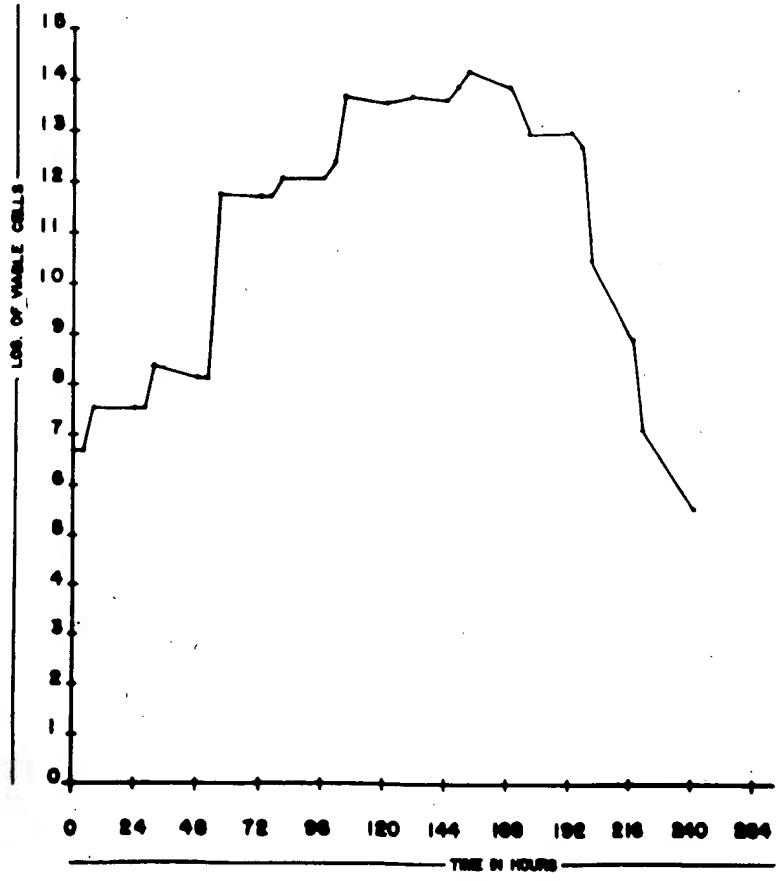

FIG. 2. *Pattern of growth of P. brasiliensis yeast phase in MMcM medium (strain C 81).*

adapted, 3-day-old cultures could be transferred to liquid MMcM medium in Erlenmeyer flasks, and abundant growth occurred if the liquid cultures were incubated at 36°C with constant agitation (120 rotations per min) on a gyratory shaker (New Brunswick Scientific). Of the six isolates studied in liquid MMcM medium, five grew such that an increase in turbidity was obvious after 60 h of incubation, with a heavy growth after 120 h of incubation. Turbidimetric readings taken from the cultures are presented in Table 2. One strain, B 339, did not grow well in the MMcM medium.

The pattern of growth of one of the isolates, C 81, was determined as described previously (1) (Fig. 2). Colony-forming units increased logarithmically during the first 108 h, after which time the stationary phase began. The stationary phase was 84 h long. The decline occurred thereafter and was relatively abrupt. By 240 h, when the experiment was terminated, colony-forming units were below the initial numbers at 0 h. This pattern is similar to the one previously described for *P. brasiliensis* grown in complex media (1, 14).

The results of this study corroborate previous findings (1) concerning the growth of *P. brasiliensis* yeast phase and indicate the points at which young cells can be obtained for physiological and antigenic studies to avoid the risk of using degenerated yeast cells.

Preliminary experiments with *P. brasiliensis* mycelial phase indicate that the mycelial phase grows well in either liquid or agar MMcM medium. This is an added advantage because any work involving comparison between the two phases requires identical substrata.

The availability of an adequate, totally defined medium for cultivation of *P. brasiliensis* will facilitate studies on the metabolic, physiological, and antigenic characteristics of this microorganism.

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